# THE TRYPANOSOMES FOUND IN A HORSE NATURALLY INFECTED IN THE GAMBIA. A DOUBLE INFECTION

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CONTENTS



## I. CONCLUSION OF A PREVIOUS PAPER

A former paper (1911) dealt with the trypanosomes found in two horses naturally infected in the Gambia. These horses were referred to as *Horse A* and *Horse B*. The former, *Horse A*, contained in its peripheral blood two forms of parasite, one long the other short; the latter, *Horse B*, presented one form of parasite only. The conclusion of the paper was as follows:—

(1) We consider the trypanosome found in *Horse B* to be *T. dimorphon, sensu* Laveran and Mesnil.

(2) The long form in *Horse A* appears to us to be *T. vivax*.

(3) As regards the short form found in *Horse A*, we do not feel justified at the present stage in assigning its position. It may be a Dimorphon-like trypanosome of low pathogenicity, or simply a modification of the long parasite of *Horse A*.

This further work, an account of which is now presented, deals solely with the two forms of trypanosome found in *Horse A*, and was directed chiefly to deciding the point as to whether the short aflagellar trypanosome was a modification of the free flagellated long form, or belonged to a different species.

## II. THE SEPARATION OF THE SHORT FORM FROM THE LONG FORM

The blood of *Horse A* contained a great preponderance of trypanosomes of long form, there being roughly speaking a thousand long forms to one short form. When the blood of *Horse A* was inoculated into laboratory animals, very varying results were obtained, according to the species of animal used. Thus, Goat 1485, inoculated directly from *Horse A* contained in its blood both long and short forms of trypanosome, and these were present in much the same proportion as in the blood of *Horse A*. On the other hand, rabbits, guinea-pigs, rats and mice inoculated with blood from *Horse A*, either failed to become infected or presented in their blood short, aflagellar trypanosomes only. There was one exception to this rule, namely, Rabbit 1467, referred to in the previous paper. In this animal both long and short forms of parasite were present in the blood during the course of the infection, the long forms predominating until the last day, when the short forms suddenly became numerous. Two mice and a rat inoculated from this rabbit became infected with short forms only. It was early apparent that, if the long form of parasite were to be maintained in animals, certain of the smaller laboratory animals (dogs, rabbits, guinea-pigs, rats, etc.) would prove of little service. Goats, however, showed themselves to be very susceptible to infection with the long form, and it has been found possible to preserve this type by passage from goat to goat. The short trypanosome was passed through the smaller laboratory animals, but proved to be, in the early stages, of remarkably low pathogenicity, especially as regards rats and mice.

The inoculation of the blood of *Horse A* into certain rabbits and guinea-pigs, resulted in infection with short forms only. It was thus possible to obtain an apparently pure infection with the short forms in certain species of animals, the long form not appearing in them.

As regards the separation of the short form from the long form, it was observed above that Goat 1485, inoculated from *Horse A*, presented both forms of parasite in its blood. A dog which was inoculated from this goat became infected, but no long forms of parasite ever appeared in its blood, short aflagellar trypanosomes only being present. A second goat, Experiment 1497, inoculated from *Horse A* presented long forms only in its blood, and it was found that a dog inoculated from it failed to become infected, either with long or short forms, as did also five rats and two mice. A goat, however, became infected on inoculation from Goat 1497, and in its blood long forms only were discovered. From this time on, the blood of the goats in which the long form has been maintained has never given rise on inoculation into the smaller animals to an infection with short forms of trypanosome.

## III. THE EVIDENCE UPON WHICH THE DIAGNOSIS OF A DOUBLE INFECTION IS BASED

Apart from the distinctive differences in morphology and measurements which are dealt with below, the following experimental evidence is now adduced to prove that two separate species of trypanosome co-existed in *Horse A*.

(*a*) The long-form strain of trypanosomes which has been kept up by passage from goat to goat for a period of nearly a year, maintains its characteristic appearance. Short forms have never been seen in the blood of the goats since the first passage. Two monkeys, *C. callitrichus* and *Macacus rhoesus*, six dogs, two cats, seven rabbits, five guinea-pigs, one brown wild rat, eighteen tame rats, and eleven mice, have been inoculated from different goats at different times. Many of the animals were re-inoculated. In none of these animals did the short form of trypanosome ever appear, nor was their blood infective on sub-inoculation into other animals of the same species.

(*b*) The short form of trypanosome which appeared in a rabbit inoculated from *Horse A*, was carried down to mice, which became infected. From one of these mice a fresh horse was inoculated. This animal became infected, and had parasites visible in its blood for one day only, and the parasites were all of a short aflagellar type. The blood of this horse was highly infective for a goat,

dogs, rabbits, guinea-pigs, rats and mice. In all the animals inoculated from this horse short trypanosomes only were observed. The most careful search failed to reveal a parasite of long form. Therefore, neither in this experimentally infected horse, which was inoculated from a mouse showing short trypanosomes only in its blood, nor in a goat inoculated from this horse, were long forms of trypanosome discovered. When one considers the facts that in *Horse A* the long form of parasite was so greatly in excess of the short, and that goats have proved themselves very susceptible to infection with this long form, one would expect that if the short parasite were merely a variation of the long form, it would, on being brought back into horses and goats, assume the long form. This did not occur.

(*c*) Many laboratory animals, after having proved themselves quite refractory to the long form of parasite, even after repeated large and small inoculations, both subcutaneously and intraperitoneally, became readily infected on being inoculated with blood containing the short form of parasite. Table I shows the effect of inoculating short-form parasites into three dogs refractory to the long form.

(*d*) Four rabbits and two white rats intraperitoneally inoculated with blood from goats infected with the long form, became infected and parasites were found in their blood for brief periods (one to ten days). The parasites were all of the long type. Efforts to convey this infection to animals of the same species by intraperitoneal and subcutaneous injection have so far failed, though repeatedly made. These facts militate very strongly against the idea that the short form of parasite is simply a modification of the long form, and it appears certain that the short form is quite a distinct species from the long form, which is *T. vivax*.

## IV. PATHOGENICITY OF *T. VIVAX* IN GOATS

In Table II are given the details regarding the pathogenicity of the long-form parasite, *T. vivax*, in a series of goats.

It will be seen that the average incubation period in a series of fourteen consecutive goats was nine days, and the average duration of the disease was thirty-one days. No goat which became infected has recovered, and no goat has failed to become infected

on inoculation with goat's blood containing *T. vivax*. The majority of the goats were observed to become paralysed in their hind legs a day or two before death and several of them had opacities of the cornea. It will be observed from this table that there is no marked diminution in the incubation period nor in the duration of the disease in the later goats, such as would indicate any definite increase in virulence of the strain. A considerable difference in this respect will be found in considering the short form.

## V. MORPHOLOGY AND MEASUREMENTS OF THE LONG FORM IN GOATS

The long parasite is free-flagellated and monomorphic. The posterior portion is broad and the body tapers gradually towards the anterior end. The nucleus lies near the centre of the trypanosome, but anterior to the broadest portion of the body; it is, as a rule, well defined, but occasionally somewhat diffuse. The blepharoplast, round and distinct, is situated either laterally close to the posterior extremity or terminally; the membrane is simple and narrow. The average measurement of a thousand of the long forms taken from four goats on twenty-two days of the disease is 21·7 $\mu$.

## VI. THE MOTILITY OF *T. VIVAX* IN GOATS

The great rapidity of movement of the trypanosome, as seen in fresh preparations of the blood, was the most striking feature observed by Ziemann, who first described it in the Cameroons, in 1903. The rapidity with which it darts across the field is most characteristic. It was observed that this *T. vivax* from the Gambia possessed this character most markedly. However, after it had been preserved for some time in goats, a distinct change was noticed as regards its motility. It gradually became slower and slower in its movements. In some cases its movement became absolutely sluggish, the parasite clinging to the corpuscles and remaining stationary, somewhat in the manner of the *pecorum* group. This change commenced about October, and the sluggishness persisted until March. During this period the motility of the parasite was very limited and by no means suggestive of the designation '*vivax*' applied to the trypanosome. Further, this slowness of movement did not appear to depend entirely upon the

laboratory temperature at which the films were usually examined as it was observed also even when the film was transferred as quickly as possible, and examined in a warm atmosphere. In March the movements became more rapid, and now, in April, the parasite has regained its former rapidity of movement and its habit of dashing across the field and out of sight.

## VII. PATHOGENICITY OF THE SHORT-FORM TRYPANOSOME IN LABORATORY ANIMALS

The experimentally infected Horse 1608 mentioned above, which was inoculated from a mouse showing only short forms, and which in turn showed only short forms in its blood, is taken as the starting point in considering the pathogenicity of the short form of parasite in rats. The appearance of short forms only in this horse, was an indication that the short form was not a derivative of the long form, and from this time on two strains of the short form have been maintained, one in rats only, derived from this horse, and another, derived from the original *Horse A*, in rabbits. In Table III are given the results of carrying on these two strains of the short form, and in addition the results of inoculations made into various other animals. A very marked increase in virulence was observed in this trypanosome after its passage through Horse 1608. The increase of virulence was most noticeable in regard to those animals which had previously proved very refractory, namely, rats and mice. For example, in the mouse from which the horse was inoculated the incubation period was eighteen days, and death had not occurred on the fifty-seventh day, whereas in the six Mice 1761, after the parasite had passed through Horse 1608, the average incubation was four days and death occurred on the thirteenth day. Thus, this parasite, already resembling *T. dimorphon, sensu* Laveran and Mesnil, not only in its appearance both in fresh and stained preparations, but also in its measurements, has further approached it in regard to its animal reactions.

## VIII. MORPHOLOGY AND MEASUREMENTS OF THE SHORT FORM IN RATS

This trypanosome is a short, non-free flagellated parasite. The protoplasm stains more deeply than that of *T. vivax*, the nucleus is central and well defined, the blepharoplast terminal or lateroterminal

and round. In dividing forms there is frequently noted, besides a considerable elongation of the parasite, the presence of a very small portion of flagellum which appears to be free. The average measurement of a thousand trypanosomes drawn from two white rats on ten days of the disease is 13·3 $\mu$. A curve showing the percentage distribution of the various lengths is in course of preparation and will be published later.

## CONCLUSION

(1) *Horse A*, naturally infected in the Gambia contained in its blood two distinct species of trypanosome.

(2) The first trypanosome, the long form, is *T. vivax*.

(3) It appears justifiable to classify the short trypanosome, taking into consideration its morphology, measurements, and the later developments of its animal reactions, as *T. dimorphon, sensu* Laveran and Mesnil.

## REFERENCE

Yorke and Blacklock (1911). 'The trypanosomes found in two horses naturally infected in the Gambia.' *Ann. Trop. Med. and Parasit.*, Vol. V, No. 3, p. 413.

Table I.—Showing the effects of inoculating the short form of parasite into dogs which had proved refractory to the long form.

| | Long Form | | Short Form | | | |
|---|---|---|---|---|---|---|
| No. of Experiment | Animal from which inoculated with long form | Result | Animal from which inoculated with short form | Incubation in days | Duration of disease in days | Remarks |
| Dog, 1609 ... | Goat 1605 reinoculated once | Parasites never seen up to 44th day | Guinea-pig 1651 B | 14 | 17 | Short forms only seen |
| „ 1612 ... | Goat 1584 | Parasites never seen up to 21st day | Horse 1608 | 9 | 28 | „ |
| „ 1661 B | „ 1584 reinoculated twice | Parasites never seen up to 33rd day | Dog 1725 | 8 | — | „ Developed acute pneumonia; killed by chloroform on account of pain |

TABLE II.—Pathogenicity of *T. vivax* in Goats

| No. of Experiment | Animal from which inoculated | Day on which parasites first found in blood | Day on which death occurred | Remarks |
|---|---|---|---|---|
| Goat 1497 ... | Horse A ... | 10th | 44th | Highest temperature recorded 107° F. |
| ,, 1559 ... | Goat 1497 ... | 11th | 64th | |
| ,, 1584 A... | ,, 1605 ... | 6th | 29th | |
| ,, 1605 ... | ,, 1559 ... | 9th | 47th | |
| ,, 1673 ... | ,, 1605 ... | 9th | 22nd | |
| ,, 1685 ... | ,, 1584 ... | 4th | 17th | |
| ,, 1733 ... | ,, 1685 ... | 7th | 16th | |
| ,, 1735 ... | ,, 1685 ... | 10th | 28th | |
| ,, 1774 ... | ,, 1733 ... | 9th | 27th | |
| ,, 1801 ... | ,, 1774 ... | 13th | 43rd | |
| ,, 1838 ... | ,, 1801 ... | 13th | 14th | |
| ,, 1858 ... | ,, 1801 ... | 11th | 16th | |
| ,, 1895 ... | ,, 1838 ... | 11th | 35th | |
| ,, 1933 ... | ,, 1895 ... | 11th | 32nd | |
| | Average ... ... | 9·6 | 31 | |

TABLE III.—The pathogenicity of the short-form trypanosome in laboratory animals

| No. of Experiment | Animal from which inoculated | Day on which parasites first found in blood | Day on which death occurred | Remarks |
|---|---|---|---|---|
| Rabbits 1494 ... | Horse A ... | 19th | 164th | |
| ,, 1784 ... | Rabbit 1494 ... | 23rd | 56th | |
| ,, 1877 ... | ,, 1784 ... | 22nd | — | Alive on 91st day |
| Rat 1650 A... | Horse 1608 ... | 6th | 78th | |
| ,, 1650 B... | ,, 1608 ... | 6th | 74th | |
| ,, 1663 A... | Rat 1650 A... | 9th | 97th | |
| ,, 1663 B... | ,, 1650 A... | 12th | 51st | |
| ,, 1663 C... | ,, 1650 A... | 7th | 45th | |
| ,, 1870 ... | ,, 1663 A... | 9th | 16th | |
| ,, 1727 A... | ,, 1663 B... | 9th | 41st | |
| ,, 1727 B... | ,, 1663 B... | 9th | 45th | |
| ,, 1909 ... | ,, 1870 ... | 7th | — | Alive 80th day |
| ,, 1919 ... | ,, 1909 ... | 10th | — | Alive 73rd day |
| ,, 1952 ... | ,, 1919 ... | 10th | 22nd | |
| ,, 1977 ... | ,, 1919 ... | 7th | 15th | |
| ,, 2004 ... | ,, 1977 ... | 4th | 8th | |
| **No. of Experiment** | **Pathogenicity in other animals** | | | |
| Goat 1657 ... | Horse 1608 ... | 10th | 19th | Paralysed in hind legs for two days before death |
| Dog 1609..... | Guinea-pig 1651 B | 14th | 17th | Dog previously used for long-form experiment. |
| ,, 1612 ... | Horse 1608 ... | 9th | 59th | |
| ,, 1688 ... | Guinea-pig 1651 B | 29th | 42nd | |
| ,, 1725 ... | ,, ,, | 12th | 13th | |
| Rabbit 1651 A | Horse 1608 ... | 12th | 43rd | |

TABLE III.—*Continued*

| No. of Experiment | Animal from which inoculated | Day on which parasites first found in blood | Day on which death occurred | Remarks |
|---|---|---|---|---|
| Guinea-pig 1651 B | Horse 1608 ... | 22nd | 34th | |
| ,, 1651 C... | ,, 1608 ... | 9th | 73rd | |
| Rat 1613 ... | Guinea-pig 1651 B | 8th | 43rd | |
| ,, 1732 A... | ,, 1651 B | 6th | 9th | |
| ,, 1732 B... | ,, 1651 B | 5th | 9th | |
| ,, 1732 C... | ,, 1651 B | 6th | 12th | |
| ,, 1732 D | ,, 1651 B | 8th | 13th | |
| ,, 1749 ... | Rat 1732 A... | 3rd | 10th | |
| ,, 1858 ... | Mouse 1764 C... | 9th | 20th | |
| ,, 1823 ... | Dog 1688 ... | 8th | 12th | |
| ,, 1860 A... | Mouse 1855 ... | 7th | 11th | |
| ,, 1860 B... | ,, 1855 ... | 6th | 9th | |
| Mouse 1756 A ... | Rat 1749 ... | 17th | 74th | |
| ,, 1756 B ... | ,, 1749 ... | 9th | 11th | |
| ,, 1761 A (1) | ,, 1732 D | 4th | 20th | |
| ,, 1761 A (2) | ,, 1732 D | 3rd | 6th | |
| ,, 1761 B (1) | ,, 1732 D | 3rd | 6th | |
| ,, 1761 B (2) | ,, 1732 D | 3rd | 11th | |
| ,, 1761 C (1) | ,, 1732 D | 4th | 22nd | |
| ,, 1761 C (2) | ,, 1732 D | 7th | 12th | |
| ,, 1764 A ... | ,, 1749 ... | — | 7th | Parasites never seen |
| ,, 1764 B ... | ,, 1749 ... | 13th | 22nd | |
| ,, 1764 C ... | ,, 1749 ... | 10th | 22nd | |
| ,, 1839 A ... | ,, 1823 ... | 10th | 17th | |
| ,, 1839 B ... | ,, 1823 ... | 8th | 10th | |
| ,, 1839 C ... | ,, 1823 ... | 9th | 14th | |
| ,, 1839 D | ,, 1823 ... | 9th | 15th | |
| ,, 1855 ... | Mouse 1839 C... | 4th | 6th | |